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Tris(dichloropropyl)phoshate, a Mutagenic Flame Retardant: Frequent Occurrence in Human Seminal Plasma

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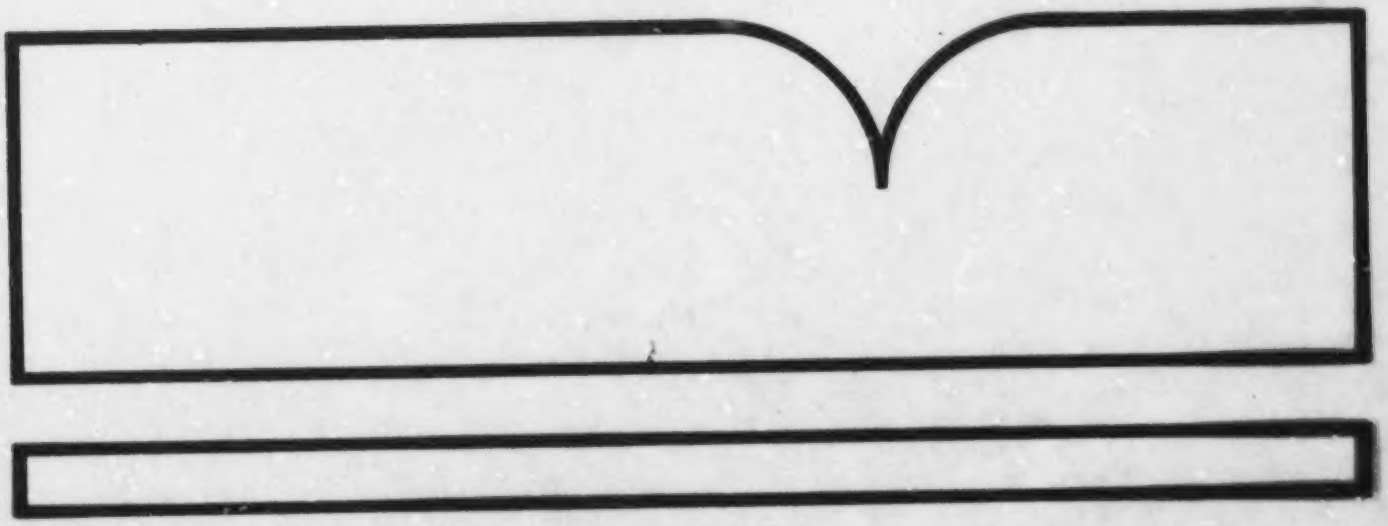

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16. ABSTRACT

Negative-chemical-ionization mass spectral screening of extracts of human seminal plasma has revealed a presence of a Cl_7 ion cluster at a mass-to-charge ratio (m/z) of 463 in a significant number of the samples examined (34 out of 123). Experiments with different gases used to generate the negative-chemical-ionization plasma indicated that the ion at m/z 463 was a chloride adduct of a Cl_6 molecule with a mass of 428 daltons. Negative-chemical-ionization mass measurement with ions from the iodoform mass spectrum used as reference peaks gave a mass of 427.882 daltons; $C_9H_{15}O_4PCl_6$ has a molecular weight of 427.883. Extraction of polyurethane foam with toluene produced an extract that consistently gave a negative-chemical-ionization spectrum containing an intense Cl_7 ion at m/z 463. The component producing this ion was isolated, and its proton nuclear magnetic resonance spectrum confirmed that it was tris(1,3-dichloro-2-propyl)phosphate, a mutagenic flame retardant. The negative-chemical-ionization screening evidence suggests that this flame retardant or its isomer tris(2,3-dichloro-1-propyl)phosphate, or both, are absorbed into the body from formulations in which they are used as flame retardants. Remedial action seems indicated to reduce human exposure to these compounds.

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The new strain-specific lethal factor could be synthesized by xD amoebas either as a result of acquiring new DNA templates or by altered expression of existing chromosomal genes. Further work is needed to distinguish between these possibilities, but the latter mechanism would be simpler in that products of symbiotic bacteria could effect an alteration of gene expression of amoebas without involving any transfer of their own DNA's. Such progressive changes in nuclear synthetic activities caused by cytoplasm occurs regularly during embryonic development of metazoans.

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25 July 1980; revised 5 November 1980

Tris(dichloropropyl)phosphate, a Mutagenic Flame Retardant: Frequent Occurrence in Human Seminal Plasma

Abstract. *Negative-chemical-ionization mass spectral screening of extracts of human seminal plasma has revealed a presence of a Cl_7 ion cluster at a mass-to-charge ratio (m/z) of 463 in a significant number of the samples examined (34 out of 123). Experiments with different gases used to generate the negative-chemical-ionization plasma indicated that the ion at m/z 463 was a chloride adduct of a Cl_6 molecule with a mass of 428 daltons. Negative-chemical-ionization mass measurement with ions from the iodoform mass spectrum used as reference peaks gave a mass of 427.882 daltons; $C_9H_{15}O_4PCl_6$ has a molecular weight of 427.883. Extraction of polyurethane foam with toluene produced an extract that consistently gave a negative-chemical-ionization spectrum containing an intense Cl_7 ion at m/z 463. The component producing this ion was isolated, and its proton nuclear magnetic resonance spectrum confirmed that it was tris(1,3-dichloro-2-propyl)phosphate, a mutagenic flame retardant. The negative-chemical-ionization screening evidence suggests that this flame retardant or its isomer tris(2,3-dichloro-1-propyl)phosphate, or both, are absorbed into the body from formulations in which they are used as flame retardants. Remedial action seems indicated to reduce human exposure to these compounds.*

Negative-chemical-ionization (NCI) mass spectral screening of minimally cleaned extracts of environmental substrates has been successful in detecting part-per-billion concentrations of substances that are oxidizing agents, alkylating agents, or both (*1*). Such NCI mass spectra can be used to selectively detect the presence of polychlorinated organic substances (*1*, *2*), phosphate and phosphothioate insecticides (*3*), polynuclear aromatic hydrocarbons (*4*), and brominated flame retardant metabolites (*5*). The technique of NCI mass spectrometry is directly analogous to gas chromatography with electron-capture detection, a major difference being that NCI mass spectrometry supplies molecularly specific information about the substances that are detected. The basis for the success of these techniques in detecting toxic substances stems from the fact that molecules that are oxidizing agents, alkylating agents, or both generally have positive electron affinities or significant anion affinities in the gas phase, or both. Biological molecules, particularly neutral lipids, which are among the most difficult substances to remove in residue cleanup procedures, generally have negative electron affinities and low NCI sensitivities. As a result, exceptionally simple cleanup procedures (*6*) can be used for NCI screening applications.

In a survey of toxic substances in seminal plasma (samples were obtained from student donors) as a function of sperm density (*7*) we frequently encountered (34 out of 123 cases) an isotope pattern indicating the presence of a cluster of seven chlorines (Cl_7) at a mass-to-charge ratio (*m/z*) of 463. The appearance of this isotope cluster was correlated with the appearance of a Cl_6 cluster at *m/z* 427 and a Cl_4 cluster at *m/z* 317. These correlations suggested that the ion cluster at *m/z* 463 was the result of the attachment of chloride to a molecule containing six chlorines with a molecular weight of 428. This presumption was strengthened when we obtained the NCI spectrum without a source of chloride, in which case only the ions at *m/z* 427 and 317 appeared. The ion at *m/z* 317 had previously been misassigned as the chloride adduct of DDMU [1-chloro-2,2-bis(*p*-chloro-phenyl)ethylene] (*6*).

Mass measurement of the ion cluster at *m/z* 463 in the negative ion mode with the chloride adduct of iodoform at *m/z* 428.691 used as a standard gave an exact mass of 462.851 daltons for the ^{35}Cl ion of the *m/z* 463 cluster. The elemental composition $C_9H_{15}O_4{}^{35}Cl_7P$ has an exact mass of 462.852 daltons.

A Cl_7 ion cluster at *m/z* 463 that corresponded in every way to the ion found in samples of human seminal plasma that had been cleaned up by steam distillation continuous liquid-liquid extraction (*6*, *7*) was a consistent occurrence in our attempts to obtain procedural blanks for a process designed to isolate planar polynuclear aromatics by adsorption on activated carbon supported on polyurethane foam. The spectra of these extracts also exhibited the ion clusters at *m/z* 427 (Cl_6) and 317 (Cl_4). As a result of this coincidence, we extracted the polyurethane foam with toluene in a Soxhlet extractor and isolated the component that gave rise to the spectrum by chromatography on alumina. The methylene chloride NCI mass spectrum of this component is illustrated in Fig. 1. Since milligram quantities of this material were available, it was possible to obtain a proton nuclear magnetic resonance (NMR) spectrum (Bruker 270-MHz Fourier transform NMR spectrometer) and a complete high-resolution, electron-impact mass spectrum. The proton spectrum of the component giving rise to the *m/z* 463 ion cluster is illustrated in Fig. 2. Proton decoupling experiments indicated that the resonances at 3.86 δ and 3.83 δ were

coupled together with a residual nonproton coupling of 5 Hz for the resonance at 4.83 δ and a coupling of 0.8 Hz for the resonance at 3.86 δ. Proton decoupling experiments also indicated that the resonance at 4.83 δ consisted of a doublet of pentuplets. The ratio of the areas of the resonances at 4.83 δ and 3.86 δ was 1/4.2. The high-resolution, electron-impact mass spectrum of this component gave mass measurements that were consistent with the elemental composition presented above. The electron-impact mass spectrum contained an intense ion at *m/z* 111, whose measured elemental composition and isotope abundance corresponded to $C_3H_5Cl_2$. This fragment is the exact difference between the molecular weight (428) and the intense ion in the NCI spectrum at *m/z* 317. From these observations it can be readily deduced that the structure of this component is tris(1,3-dichloro-2-propyl)-phosphate.

The widespread exposure to tris(1,3-dichloro-2-propyl)phosphate in the environment is evidenced by the fact that it was difficult for us to obtain acceptable procedural blanks for this compound. All of the positive samples had ion intensities at least ten times those of quality-control procedural blanks. We removed this compound from our reagent-grade water by repeated extraction with toluene. It was removed from organic solvents, particularly hexane, by extraction with concentrated sulfuric acid.

Fyrol FR2 is the commercial name for tris(1,3-dichloro-2-propyl)phosphate. It has been used as a flame retardant for fabrics including Dacron (*8*) and is also used as a flame retardant for polyurethane foam (*9*), which accounts for our ability to isolate the compound from that source.

Having the isolated standard of tris(1,3-dichloro-2-propyl)phosphate, it was possible to determine detection limits and to calibrate the response for detection of this component against a standard, decafluorotriphenylphosphine. The detection limit for the tris(dichloropropyl)phosphate was 10 ng (signal-to-noise ratio, 5:1), and the calibration against a standard amount of the fluorophenylphosphine standard was linear over the range 10 ng to 1 μg. Since we had used decafluorotriphenylphosphine as a sensitivity standard in the seminal plasma screening, with these data it was possible to estimate that the seminal plasma samples that were positive in those experiments contained the tris(dichloropropyl)phosphate in concentrations ranging from 5 to 50 parts per billion. Because lipophilic components

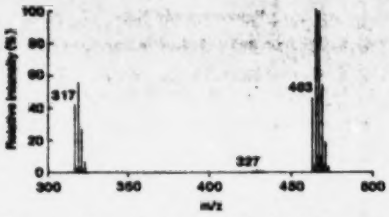

Fig. 1. Negative-chemical-ionization mass spectrum (methylene chloride/isobutane) of tris(1,3-dichloro-2-propyl)phosphate (isolated from polyurethane foam).

are selectively partitioned into the sperm cells as compared to the plasma, these concentrations may be significant from a toxicological point of view.

The NCI detection system that we use is not isomer-specific. The seminal plasma samples that gave an ion at *m/z* 463 with an exact mass corresponding to $C_9H_{15}O_4{}^{35}Cl_7P$ could have contained either tris(1,3-dichloro-2-propyl)phosphate or tris(2,3-dichloro-1-propyl)phosphate, both of which are commercial flame retardant additives.

These compounds might not have been detected in gas chromatography–mass spectrometry screening. Both of the isomeric tris(dichloropropyl)phosphates decompose under ordinary gas chromatography conditions (*10*). Using isothermal (197°C) electron-capture gas chromatography (Varian 1400, 1.8 by 2 mm inside diameter, 1.95 percent QF-1 and 1.5 percent OV-17 on 80 to 100 mesh Gas-Chrom Q), we found that tris(1,3-dichloro-2-propyl)phosphate gave eight peaks with retention times relative to aldrin of 1.39, 1.63, 3.26, 3.58, 4.74, 6.79, 8.67, and 10.81. The two largest peaks, those with relative retention times of 6.79 and 8.67, appeared in the electron-capture

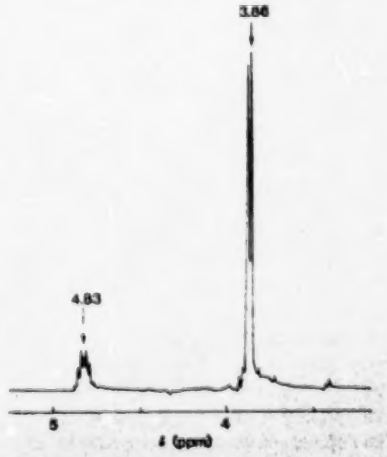

Fig. 2. Proton NMR spectrum (270 MHz) of tris(1,3-dichloro-2-propyl)phosphate ($CDCl_3$ solvent).

gas chromatography runs for the seminal plasma extracts whose NCI spectra indicated the presence of tris(1,3-dichloro-2-propyl)phosphate.

The compounds tris(dichloropropyl)-phosphate, tris(dibromopropyl)phosphate, and dibromochloropropane are closely related chemically. All three are known to be mutagenic (*11*), and the latter two are known to be sterilifactants (*12*). Evidence suggests that there may have been a significant decline in fertility in the United States over the last three decades (*7*, *13*). It is conceivable that the presence of chlorinated substances, such as Fyrol, in seminal fluid could have a bearing on this decline. It is known that flame retardant chemicals of this class can be absorbed into the body from treated fabrics over a short time span (*5*); it should be possible to absorb these compounds from sources such as flame retardant-treated consumer products. In view of the toxicity of these chemicals, it would seem prudent to limit human exposure to them to an absolute minimum.

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14. This work was supported by contract N1H-ES-79-2 from the National Institute of Environmental Health Sciences and in part by a grant from the same source. We thank S.-Y. Tang, M. Whitaker, and Y. Tondeur for assistance.
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Fyrol FR2 is the commercial name for tris(1,3-dichloro-2-propyl)phosphate. It has been used as a flame retardant for fabrics including Dacron (*8*) and is also used as a flame retardant for polyurethane foam (*9*), which accounts for our ability to isolate the compound from that source.

Having the isolated standard of tris(1,3-dichloro-2-propyl)phosphate, it was possible to determine detection limits and to calibrate the response for detection of this component against a standard, decafluorotriphenylphosphine. The detection limit for the tris(dichloropropyl)phosphate was 10 ng (signal-to-noise ratio, 5:1), and the calibration against a standard amount of the fluorophenylphosphine standard was linear over the range 10 ng to 1 μg. Since we had used decafluorotriphenylphosphine as a sensitivity standard in the seminal plasma screening, with these data it was possible to estimate that the seminal plasma samples that were positive in those experiments contained the tris(dichloropropyl)phosphate in concentrations ranging from 5 to 50 parts per billion. Because lipophilic components

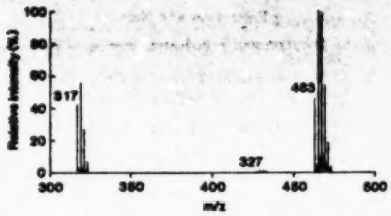

Fig. 1. Negative-chemical-ionization mass spectrum (methylene chloride/isobutane) of tris(1,3-dichloro-2-propyl)phosphate (isolated from polyurethane foam).

are selectively partitioned into the sperm cells as compared to the plasma, these concentrations may be significant from a toxicological point of view.

The NCI detection system that we use is not isomer-specific. The seminal plasma samples that gave an ion at *m/z* 463 with an exact mass corresponding to $C_9H_{15}O_4{}^{35}Cl_7P$ could have contained either tris(1,3-dichloro-2-propyl)phosphate or tris(2,3-dichloro-1-propyl)phosphate, both of which are commercial flame retardant additives.

These compounds might not have been detected in gas chromatography–mass spectrometry screening. Both of the isomeric tris(dichloropropyl)phosphates decompose under ordinary gas chromatography conditions (*10*). Using isothermal (197°C) electron-capture gas chromatography (Varian 1400, 1.8 by 2 mm inside diameter, 1.95 percent QF-1 and 1.5 percent OV-17 on 80 to 100 mesh Gas-Chrom Q), we found that tris(1,3-dichloro-2-propyl)phosphate gave eight peaks with retention times relative to aldrin of 1.39, 1.63, 3.26, 3.58, 4.74, 6.79, 8.67, and 10.81. The two largest peaks, those with relative retention times of 6.79 and 8.67, appeared in the electron-capture

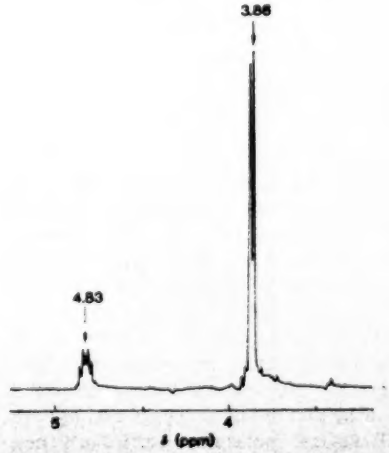

Fig. 2. Proton NMR spectrum (270 MHz) of tris(1,3-dichloro-2-propyl)phosphate ($CDCl_3$ solvent).

gas chromatography runs for the seminal plasma extracts whose NCI spectra indicated the presence of tris(1,3-dichloro-2-propyl)phosphate.

The compounds tris(dichloropropyl)-phosphate, tris(dibromopropyl)phosphate, and dibromochloropropane are closely related chemically. All three are known to be mutagenic (*11*), and the latter two are known to be sterilifactants (*12*). Evidence suggests that there may have been a significant decline in fertility in the United States over the last three decades (*7*, *13*). It is conceivable that the presence of chlorinated substances, such as Fyrol, in seminal fluid could have a bearing on this decline. It is known that flame retardant chemicals of this class can be absorbed into the body from treated fabrics over a short time span (*5*); it should be possible to absorb these compounds from sources such as flame retardant-treated consumer products. In view of the toxicity of these chemicals, it would seem prudent to limit human exposure to them to an absolute minimum.

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